*International Journal of Engineering & Technology*, 7 (3.36) (2018) 108-112

International Journal of Engineering & Technology

*Website: www.sciencepubco.com/index.php/IJET*

***Research paper***

# Investigation of Pulse Electric Field Effect on HT29 Cell Alignment Properties Cultured on Laminin Micro-Patterned Surface

**Hassan Buhari Mamman[1, 2*], Muhammad Mahadi Abdul Jamil[2], Mohammed Ahmed Bawa[3] and Mohamad Nazib Adon[2]**

[1]*Department of Electrical and Electronics Engineering, Faculty of Engineering and Engineering Technology, Abubakar Tafawa Balewa University Bauchi, Bauchi, Nigeria*
[2]*Biomedical Engineering Modeling and Simulation (BIOMEMS) Research Group, Department of Electrical and Electronics Engineering, Universiti Tun Hussein Onn Malaysia, Johor, Malaysia*
[3]*Department of Mechanical/Production Engineering, Faculty of Engineering and Engineering Technology, Abubakar Tafawa Balewa University Bauchi, Bauchi, Nigeria*
**Corresponding author E-mail: buharimamman2007@yahoo.com*

**Abstract**

Pulse electric field (PEF) is a way of generating transient holes in the cell membrane. This is achieved by exposing the cell to a high voltage electric field of usually of short duration. The application of PEF to the cell cannot only open pores in the cell membrane but can also affect the cell physiology. Extracellular matrix protein is the major regulator of many cellular functions such as proliferation, adhesion and migration. PEF was also found to modulate these cellular behaviours. However, a combined influence of PEF and ECM on cellular behaviour which could further enhances the cellular processes for wound healing application via directed cell migration has not been investigated. Therefore, the aim of this study is to examine the effect of PEF in combination with ECM on the cell guidance and self-assemble monolayer of HT29 cell line. Cell alignment was investigated via micro-contact printing techniques. The results of the study have shown that PEF has improved the HT29 cell alignment and elongation by more than 40%. Since tissue development in multicellular organisms in the course of wound healing depends on the cell adhesion process which can be influence by electrostatic charges. Therefore, manipulation of substrate charge by patterning the substrate and application of PEF to enhance cell adhesion is a promising scheme that can regulate cell guidance for wound healing application.

***Keywords***: *Cell membrane; cell migration; electric field; wound healing.*

## 1. Introduction

Extracellular matrix (ECM) protein is the leading controllers of numerous cellular functions such as cell division, cell to ECM adhesion, cell communication and cell to cell adhesion [1]. Moreover, ECM is the major regulator of cell migration and plays a vital function in the formation of cell shape. The major impairment in wound healing process for diabetic patient is poor cell migration. Since wound healing process are generally characterized by cell migration , proliferation and cell differentiation Therefore, understanding the process that controls cell function such as proliferation adhesion and migration is very significant for wound healing, tissue engineering application and in the development of new tissue in vivo [2]. Laminin, fibronectin, and collagen are the major components of ECM protein. Each of this protein binds to a specific integrin molecule. The secretion of this protein in the course of development and the level of their expression control many cellular functions in development [3].

There are different methods that are used for the deposition of proteins on a surface. This includes plasma-induced micro-patterning, soft lithography, ultraviolet radiation micro-patterning and micro-contact printing methods [4]. However, micro-contact printing (MCP) is chosen in this paper because it offers a cheap and simple surface patterning technique. MCP does not denaturing the proteins as in the case of others method [5]. Additionally, a sub-unit of 0.1um patterns of protein can be produced with MCP [5]. In this paper, MCP was used to produce 25, 50 and 100µm micro-pattern of Laminin protein. This is done with aim to determine the MCP dimensions that would induce the best degree of HT29 cell alignment. Subsequently, the best MCP dimension was then used to further investigate the cellular alignment under the influence of pulse electric field (PEF).

## 2. Materials and methods

### 2.1. Cell culture

The HT29 cells line were grown in a 25cm$^2$ culture flask (Life Science, Korea) as a monolayer in RPMI 1640 medium supplemented with 10% Fetal bovine serum (FBS) and 1% penicillin-streptomycin (antibiotic), which are all products of Gibco, USA. The details of the cell culture and subculture procedure can be found in [6].

**Copyright © 2018 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.**

### 2.2. Micro-contact printing technique

The Polydimethylsiloxane (PDMS) stamps of different width (25, 50 and 100µm) and glass coverslips were washed with 70 % ethanol and let to dry totally in a bio-safety cabinet before commencing the MCP procedure. The Laminin protein was diluted to a concentration of 50µg/ml using Phosphate Buffer Saline (PBS) [7]. Glass coverslips were then micro-contact printed with Laminin protein as follows: stamp was inked by dipping into the Laminin solutions for 60 seconds [8]. The stamp was then removed from the protein solution and allowed to dry in air for 90 seconds.
The dried stamp was then placed in contact with the glass coverslip or substrate and pressed lightly using another glass coverslip for 60 seconds. The process was repeated for the different stamp width (25, 50 and 100µm). Thus, this allowed the glass coverslips to be patterned with 25, 50 and 100µm width of the Laminin coated tracks separated by 25, 50 and 100µm width of uncoated tracks respectively. A protein free coverslip was used as a control.

### 2.3. Protocol for cell electroporation

In this study, the commercial electroporator ECM830 made from BTX Harvard apparatus was used for exposing the HT29 cell line to electric field while in suspension before seeding onto the substrates. The low voltage (LV) mode of the BTX ECM 830 electroporator at a voltage of 240V with a 4mm cuvette was used to achieve a 600V/cm electric field strength earlier optimized for HT29 cell line [9]. At the outset, cells are detached using the subculture procedures described in [10].
After neutralizing the effect of the detaching enzyme, 800µl of cells suspension at a concentration of $1\times10^5$ cells/ml was poured into a 4mm cuvette and then placed in BTX ECM 830 electroporator chamber. Electroporation was executed with an electric field of 600V/cm intensity for 500µs duration. Immediately after electroporation, the cuvette was moved to biosafety hood.

### 2.4. Protocol of plating cells on glass coverslips

The glass coverslips that were micro-contact printed with the three different stamp grating of the Laminin were placed, one in each well of a 6-wells plate. As control, a protein free glass coverslip was also placed in another well. Subsequently, 2ml of complete growth media was added to each well. Next, 0.1ml of HT29 cells at concentrations of $1\times10^5$ cells/ml were discharged in each well. The plate was then cultured in an incubator at 37°C and 5% $CO_2$ for 48 hours.
Following 48 hours in culture, cells were then imaged with standard phase contrast microscope. Subsequently, the best pattern identified was seeded with electroporated HT29 cells of same concentration as the untreated cells and were also incubated at the same physiological conditions for further observation.

### 2.5. Image acquisition and measurement

Forty eight hours after seeding, Images were acquired using Nikon TS100 Inverted microscope equipped with Dino camera and Dinocapture software2.0. Five Images from five different fields of view were acquired from each coverslips (control, 25, 50 and100µm stamp size). Using angle and line measurement tool in Dinocapture software2.0, the angle of alignment and cell elongation of 50 cells from each coverslip were measured and analysed. Cell alignment to the patterns was computed by measuring the angle between the cell longest axes in relation to the stamp pattern. Pattern that gives the best cell alignment was identified each experiment was repeated three times for better statistical analysis.

## 3. Results and discussion

### 3.1. Stamp construction and micro-contact pattern printing

Figure 1 depicts the images of the PDMS stamps of different width (25, 50 and 100µm) under microscope. The PDMS stamps were used for the creation of the Laminin patterns on the glass coverslips. The various patterns were successfully produced as shown in Figure 2 for the Laminin protein.

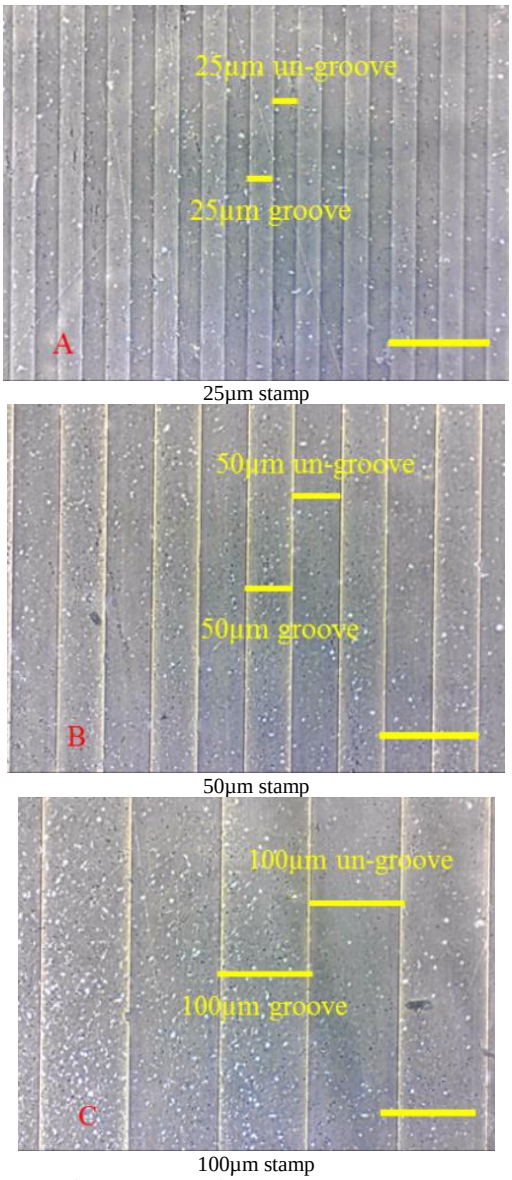
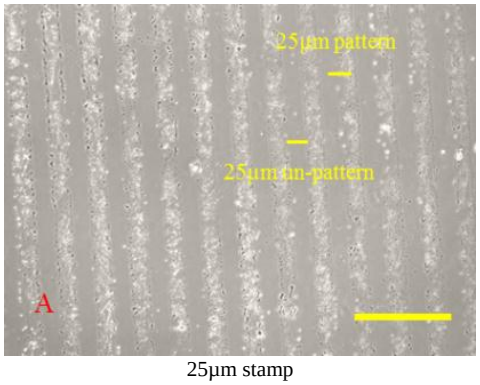
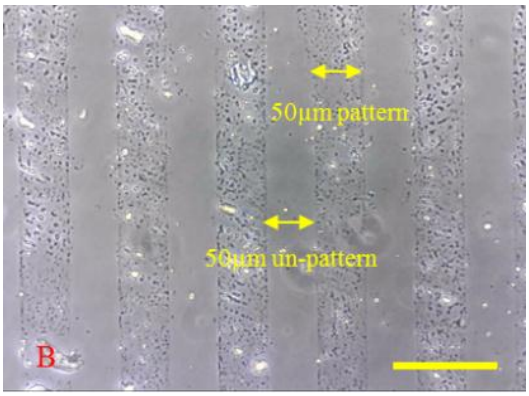
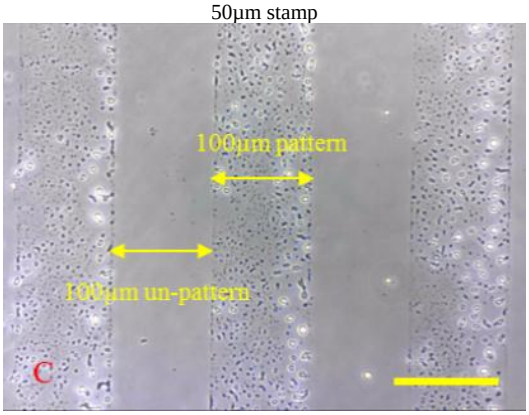



**Fig. 1** Images of PDMS stamp of various size under microscope. Scale bar = 100µm






50µm stamp

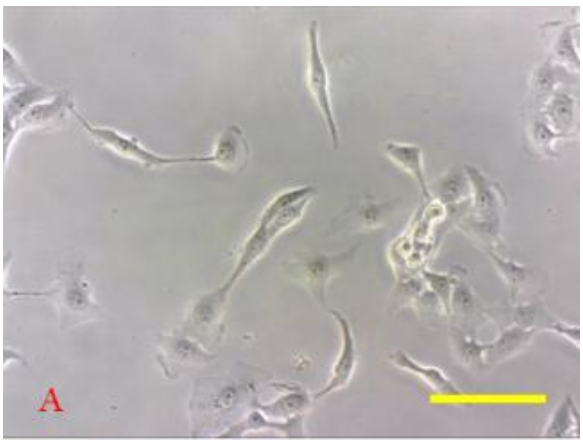



100µm stamp

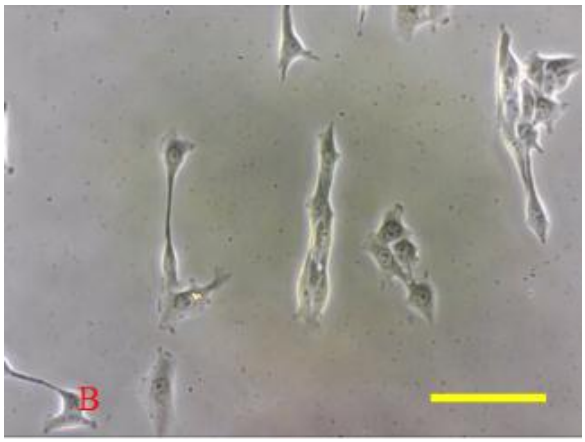
**Fig. 2** Images of glass coverslips micro-contact printed with different stamp size of Laminin protein. Scale bar = 100µm.

Figure 3 shows the images of HT29 cell line after 48 hours of seeding on the 25, 50 and 100µm width micro-contact printed substrates of Laminin, and the control group. All substrates were seeded with the same number of cell suspension. The qualitative analysis of the images has revealed that HT29 cell line aligned most readily on 100µm of Laminin pattern, followed by 50µm pattern. The cells poorly aligned on 25µm pattern. Conversely, the cells have shown no alignment on the control group with no definite pattern. Similarly, cell elongation reflected the result of cell alignment with 100µm pattern of Laminin showing highest cell elongation followed by 50µm and then 25µm.

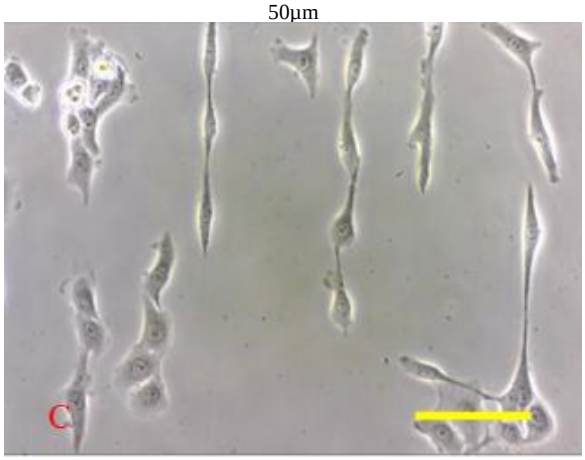
The result of angular alignment would be interpreted as; an angle of 45º and above is signifying that orientation of cell to the pattern is indiscriminate, that is, no alignment [9], [8]. While, an angle of less than 45º is suggesting that cell aligns to the pattern with zero degrees representative a complete alignment to the pattern [11]



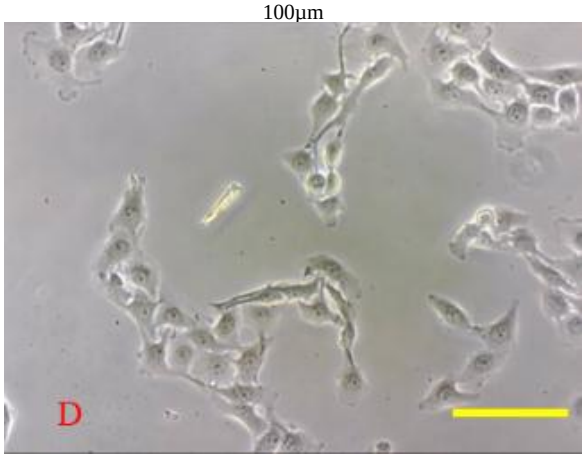

25µm




50µm




100µm




Control

**Fig. 3** Images of HT29 cell line seeded on the glass coverslips micro-contact printed with different stamp size of Laminin protein after 48 hours. Scale bar = 100µm

### 3.2. Measurement of cell alignment

Table 1 shows the data obtained from quantitative analysis of the mean angle of HT29 cell alignment on the Laminin protein. This includes the different patterned substrates (25, 50 and 100µm) and the control substrate. The angular alignment of 50 cells from different field of views, were measured and analysed in each case. The quantitative analysis of the data obtained on the patterned substrates has shown that HT29 cell line aligned most readily on the 100 and 50µm stamp patterned substrate with a mean angle of alignment of 4.5º ± 0.96 and 5.0º ± 1.45 respectively as shown in Table 1. Whereas, the HT29 cell line has revealed poor alignment on the 25µm stamp patterned with a mean angular alignment of 30.4º ± 12.2. In addition, the HT29 cell has shown no alignment on control coverslip with average angle of 54.5º ± 6.01. Laminin was found to promote the differentiation of epithelium cell [12].

Hence this could the reason for the better alignment of HT29 cells in Laminin pattern.

**Table 1** Angle of alignment for HT29 cell line seeded on coverslip micro-contact printed with ECM protein after 48 hours of seeding

| Treatment | Stamp size | Average angle of alignment ± Standard deviation (SD) |
|---|---|---|
| Laminin | 25µm | 30.3° ± 12.20 |
| | 50µm | 5.0° ± 1.45 |
| | 100 µm | 4.5° ± 0.96 |
| | Control | 54.5° ± 6.01 |

In all experimental data obtained with respect to cells angle of alignment, the data were found to be normally distributed ($P > 0.05$) and therefore one-way analysis of variance (ANOVA) followed by Post Hoc Turkey HSD test was used in analysing the data for statistical significance difference. The statistical results revealed that there is no significant difference between 50µm and 100µm stamp sizes of Laminin ECM protein ($P > 0.05$). However, there was a significant difference in angle of alignment between 100µm-25µm, 100µm-control 50µm-25µm, and 50µm-control substrates of the Laminin protein ($P < 0.05$ in all case).

### 3.3. Measurement of cell elongation or cell length

The cell length of 50 cells from different field of views, were measured and analysed in each case. The result of cell elongation would be interpreted as; the longer the cell length the better the alignment of the cell to the pattern [8]. The result of cells elongations reflects the result of cell alignment, cells that shown best alignment revealed the highest elongation. In other words, cell elongation is proportional to the cell alignment. Table 2 shows the data obtained from quantitative analysis of the mean cell length of all the ECM protein used on the different patterned substrates (25, 50 and 100µm), and control substrate. The mean cells length on the 100, 50 and 25µm stamp patterned substrates, were 103.2µm ± 19.69, 90.5µm ± 13.95 and 65.3µm ± 12.86 respectively. Whereas, the mean cells length on the control substrate was 39.6µm ± 5.90.

**Table 2** Average length of HT29 cell line seeded on coverslip micro-contact printed with ECM protein after 48 hours.

| Treatment | Stamp size | Average cell length (µm) ± SD |
|---|---|---|
| Laminin | 25µm | 65.3 ± 12.86 |
| | 50µm | 90.5 ± 13.955 |
| | 100 µm | 103.2 ± 19.69 |
| | Control | 39.6 ± 5.9 |

The result of the study showed that HT29 is most readily aligned to the 50 and 100µm stamp size. This could be connected to the cell size, whereby for smaller stamp size (relative to the average cell length of the HT29 cell line), the cell could not distinguish between the patterns protein lines and un-pattern protein lines, in which case the cell could overlap [7]. Whereas for stamp size greater than the average cell length of the HT29 cell line, as in the case of 50 and 100µm stamp size, the cells could easily distinguish between the protein pattern lines and the protein un-pattern lines. Hence, they could easily follow the guidance cues [8]. The results obtained in this research (results without PEF treatment) are qualitatively in agreement to that of [7], [8], [11] that also demonstrate that Osteoblast, Keratinocyte cell line and MG63 bone cell respectively, are most readily aligned to 50 and 100µm. In order to have better assumption we need to further investigate the cell alignment and elongation with PEF.

### 3.4 Influence of PEF on cell alignment and cell elongation

Cell alignment on 100µm stamp pattern of Laminin was the best. Hence, it was used to investigate the influence of PEF on the HT29 cell line alignment on the MCP surface. The cells were electrically treated as described in section 2.3 before plating on the patterned substrates of 100µm width stamp. The cells were cultured for 48 hours with the same number of cells as in the case of the untreated cells and under the same physiological conditions. Figure 4 shows the images of HT29 cell line after 48 hours of seeding on 100µm stamp pattern of Laminin.

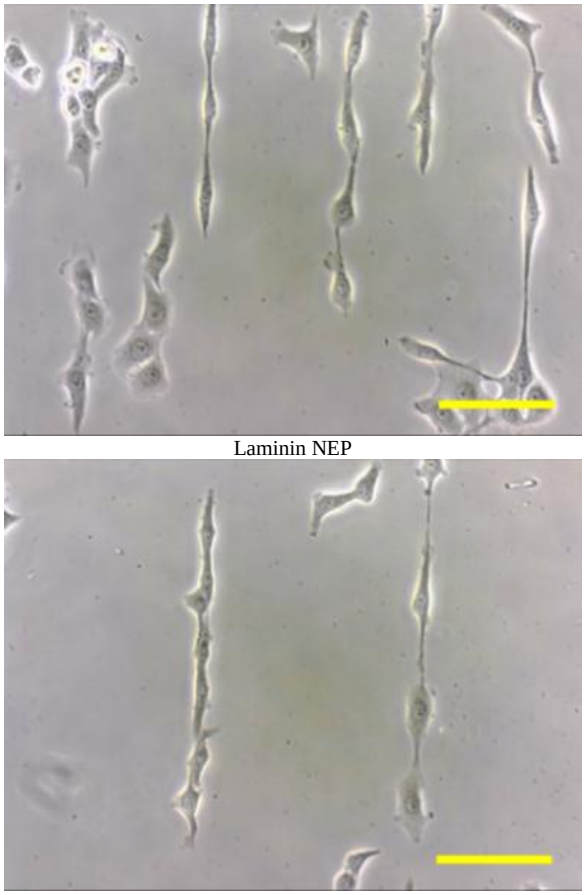



**Fig. 4** Images of HT29 cell line on 100µm micro-contact printed substrates of Laminin. Scale bar = 100µm

The results of the HT29 cell alignment and elongation, when they are exposed to PEF, on the 100µm pattern of Laminin has improved significantly. The angular alignments of HT29 cell was 3.8° ± 0.94 for the 100µm pattern of Laminin under the influence of PEF treatment. On the other hand, the cell lengths of HT29 cells were 113.7µm ± 16.85 for the 100µm pattern of Laminin under PEF treatment. Under most situations, cell membrane has a net negative charge, and when a DC electric field is applied to a cell, the cells are readily moved by electrophoresis (Gerard, 2004). Therefore, by generating positive voltages at micro-patterned surface, electrophoresis can be used to attract and pattern cells [13]. Thus, this could be the reason the EP cell has better alignment than the NEP cells.

**Table 3** Treated and Untreated HT29 cell line alignment and Elongation on 100µm micro-contact printed substrates of Laminin.

| Treatment | average angle of alignment (degree) ± SD | average cell length (µm) ± SD |
|---|---|---|
| EP | 3.8 ± 0.94 | 113.7 ± 16.85 |
| NEP | 4.5 ± 0.96 | 103.2 ± 19.69 |

The data obtained from PEF treatment revealed that there was an improvement on the alignment of HT29 cell line on 100µm patterned of Laminin which decreased from 4.5° ± 0.96 to 3.8° ± 0.94. The result is also statistically significance ($P = 0.006 < 0.05$) with a mean difference of 0.7°. Similarly, the cell elongation under PEF on the 100µm patterned of Laminin increased from 103.2µm ±

19.69 to 113.7µm ± 16.85 which is also statistically significant (P = 0.03 < 0.05) with a mean difference of 10.5µm.

The result has shown that PEF has great influence on the HT29 cell line alignment and elongation. This could be that PEF stimulated the integrin and cadherin which are the molecules responsible for cell-cell and cell-ECM adhesion [14] [15]. In addition, it could be that the PEF modulated the electrostatic charges on the cell which is also significant and accountable in cell adhesion and alignment [16]. The HT29 cell have shown strong tendency to direct their movement by contact guidance along the edge of the protein pattern cues. It is possible that the protein patterns provide a suitable surface for the adhesion of the cell lamellipodia and filopodia thereby instigated their projection along the patterns. In addition, the PEF could have further stimulated the filopodia and the cell signaling pathway and facilitated the cell to aligned better on the pattern cues as compared to the cell that are not exposed to the PEF. Growth and repair in multicellular organism depends solely on cell adhesion which is also affected by electrostatic charges on the cell surroundings [17].

## 4. Conclusion

In this paper, the influence of PEF on micro-contact printing technique of HT29 cell has been studied. The HT29 cells were successfully cultured on Laminin patterned surfaces. The cells were found to aligned and elongated most readily on the 100 and 50µm wide stamp pattern of all the Laminin ECM protein. Best cell alignment and elongation was revealed on the 100µm of stamp width of the Laminin protein. Additionally, when the cells were exposed to PEF before seeding on the patterned surface, the cell alignment and elongation was greatly enhanced. Since tissue development in multicellular organisms in the course of wound healing process depends on the cell adhesion process which can be influence by electrostatic charges [17]. Therefore, manipulation of substrate charge, for instance, by patterning the substrate or by application of external electric field to enhance cell adhesion, is a promising scheme that can control cell assembly and migration in wound healing applications [18].

## Acknowledgement

The authors wish to thank the Tertiary Education Trust Fund (TETFund) for sponsoring the paper publication through Abubakar Tafawa Balewa University Bauchi, Nigeria. We also wish to thank the University Tun Hussein Onn Malaysia for providing the equipment and materials used in conducting the research.

## References

[1] Raab, M., Daxecker, H., Edwards, R. J., Treumann, A., Murphy, D. & Moran, N., "Protein interactions with the platelet integrin αIIb regulatory motif", *Proteomics*, Vol.10, No.15, (2010), pp. 2790-2800.

[2] Metcalfe, A. D. & Ferguson, M. W., "Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration", *Journal of the Royal Society Interface*, Vol.4, No.14, (2007), pp. 413-437.

[3] Han, F., Gilbert, J. R., Harrison, G., Adams, C. S., Freeman, T., Tao, Z. & Norton, P. A., "Transforming growth factor-β1 regulates fibronectin isoform expression and splicing factor SRp40 expression during ATDC5 chondrogenic maturation", *Experimental Cell Research*, Vol.313, No.8, (2007), pp. 1518-1532.

[4] Jamil, M. M. A., Youseffi, M., Britland, S. T., Liu, S., See, C. W., Somekh, M. G. & Denyer, M. C. T., "Widefield Surface Plasmon Resonance Microscope: a Novel Biosensor Study of Cell Attachment to Micropatterned Substrates" *In 3rd Kuala Lumpur International Conference on Biomedical Engineering*, (2007), pp. 334-337.

[5] Perl, A., Reinhoudt, D. N. & Huskens, J., "Microcontact printing: limitations and achievements", *Advanced Materials*, Vol.21, No.22, (2009), pp. 2257-2268.

[6] Mamman, H. B., Jamil, M. M. A., & Adon, M. N., "Studying the influence of electroporation on HT29 cell line interaction with micro-patterned extracellular matrix protein (fibronectin)", In Control System, Computing and Engineering (ICCSCE), (2016), pp. 292-297.

[7] Mamman, H. B., Jamil, M. M. A. & Adon, M. N., "Investigation of Electroporation Effect on HT29 Proliferation Rate and Cell Size Plated on Fibronectin Coated Substrate", *Advance Science Letter*, Vol.24, No.6, (2018), pp4387-4390.

[8] Sefat, F., Denyer, M. C. T. & Youseffi, M., "Imaging via widefield surface plasmon resonance microscope for studying bone cell interactions with micropatterned ECM proteins", *Journal of Microscopy*, Vol.241, No.3, (2011), pp. 282-290.

[9] Rahman, N. A. A., Mamman H. B. & Jamil, M. M. A., "An overview: Investigation of Electroporation technique on cell properties culture on the micro-patterned surface", *Jurnal Teknologi*, Vol.77, No.6, (2015), pp 61-65

[10] Mamman, H. B., Jamil, M. M. A. & Adon, M. N., "Optimization of electric field parameters for HT29 cell line towards wound healing application", *Indian Journal of science and Technology*, Vol.9, No.46, (2016), pp1-7

[11] Khaghani, S., Sefat, F., Denyer, M., & Youseffi, M., "Alignment of rat primary chondrocyte cells to collagen type-i, fibronectin and laminin", *Journal of Anatomy*, Vol.213, No.3, (2008), pp. 351.

[12] Bradshaw, M., Ho, D., Fear, M. W., Gelain, F., Wood, F. M. & Iyer, K. S., "Designer self-assembling hydrogel scaffolds can impact skin cell proliferation and migration", *Scientific Reports*, Vol.*4*, (2014), pp. 1-6.

[13] Iwai, R., Nemoto, Y. & Nakayama, Y. "The effect of electrically charged polyion complex nanoparticle-coated surfaces on adipose-derived stromal progenitor cell behavior", *Biomaterials*, Vol.34, No.36, (2013), pp. 9096-9102.

[14] Gumbiner, B. M., "Regulation of Cadherin-mediated Adhesion in Morphogenesis", *Nature Reviews Molecular Cell Biology*, Vol.6, No.8, (2005), pp. 622-634.

[15] Théry, M. "Micropatterning as a tool to decipher cell morphogenesis and functions", *Journal of Cell Science*, Vol.123, No.24, (2010), pp. 4201-4213.

[16] Sefat, F., Youseffi, M., Khaghani, S. A., Soon, C. F. & Javid, F., "Effect of Transforming Growth Factor-β3 on mono and multilayer chondrocytes", *Cytokine*, Vol.*83*, (2016), pp. 118-126.

[17] Funk, R. H. & Monsees, T. K., "Effects of electromagnetic fields on cells: physiological and therapeutical approaches and molecular mechanisms of interaction", *Cells Tissues Organs*, Vol.182, No.2, (2006), pp. 59-78.

[18] Taghian, T., Sheikh, A. Q., Kogan, A. B., & Narmoneva D., "Harnessing electricity in biosystems - A functional tool for tissue engineering applications", *Austin Journal of Biomedical Engineering*, Vol.1, No.5, (2014), pp. 1-5.